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# Brassinosteroid Combined With Indolbutyric Acid in Blueberry Micropropagation

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**Abstract**

The lack of availability of good quality seedlings for blueberry cultivation is an obstacle in the market, preventing the increase of production and cultivated areas. In order to improve rooting of blueberry *in vitro*, different concentrations of BIOBRAS 16® associated with indolbutyric acid in blueberry micropropagation were evaluated. For such a purpose, the Wood Plant Medium (WPM) culture medium plus the following plant regulators: 0.1, 0.3 and 0.5 mg $L^{-1}$ brassinosteroids (BIOBRAS 16®) in conjunction with indolbutyric acid (IBA) concentrations of 1.3 and 5 mg $L^{-1}$, with four replications were taken into account. At the end of 82 days of cultivation and development of the explants in these culture media, the following response variables were assessed: callus percentage (CP), callus diameter (CD), rooting percentage (RP), number of shoots (NS), number of leaves (NL), shoot length (SL), root length (RL) and fresh mass of shoots (FMS). CP was found to be stimulated in so far as concentrations of BIOBRAS 16® and IBA increased up to 0.5 mg $L^{-1}$ and their diameter increased at concentrations of 3.0 and 5.0 mg $L^{-1}$ for IBA. Thus, it is concluded that a combination of 0.3 mg $L^{-1}$ BIOBRAS 16® combined with IBA concentrations of 3.0 and 5.0 mg $L^{-1}$ contributes to promote root growth and rises in leaf number and fresh mass of micro-propagated blueberry. The use of a 0.3 mg $L^{-1}$ of BIOBRAS 16® associated with concentrations of 3.0 and 5.0 mg $L^{-1}$ of IBA showed a high percentage of root formation in blueberry.

**Keywords:** *Vaccinium ashei* Reade, tissues culture, growth regulators

## 1. Introduction

The commercial blueberry takes place especially in North America (USA and Canada), Europe (Poland and Germany) and countries located in the South Hemisphere (Chile, Argentina, Uruguay, and Australia). Its production is increasing yearly owing to popularization of its medicinal properties, such as number of antioxidants besides being largely utilized in cuisine and gastronomy (Schuch et al., 2007; Cantuarias-Avilés, 2010; Cantuarias-Avilés et al., 2014; Baba et al., 2018; Kalt et al., 2019).

The climate of Southern Brazil turns out to be favorable to blueberry production. Nevertheless, different types of soils and climates reported in conjunction with the difficulty of crop management, slow growth and lack of seedlings supply to the market come to being the main factors that scupper expansion of blueberry cultivation all over the country (Souza et al., 2011; Peña et al., 2012).

Commercial production of blueberry has been made by means of rooting of stakes, once the germination rates of seeds is quite low (Pasqualini et al., 2016; Baba et al., 2018). However, such a method is conducive to disadvantages such as the need of a long period for seedling production along with the impossibility of assuring phytosanitary quality (Erig & Schuch, 2005). Another problem refers to difficulties of rooting, which has been reported in some cultivars in such a manner as to compromise propagation (Baba et al., 2018). In this particular case, micropropagation turns out to be advantageous because it provides high quality of plants with sanitary quality by demanding fewer plant materials. In order to galvanize *in vitro* rooting it is pivotal to add to tissue cultures growth regulators such as auxins acting in the formation of adventitious roots, activation of vascular cambium cells and plant growth (Kerbauy, 2008; Taiz & Zeiger, 2017). Among auxins, indolbutyric acid (IBA) is one of the most used because it deals with a phytohormone that possesses such a cell enlargement capacity with its action on cell growth triggered by growth stimulation of shoots and leaves.

Another phytohormone of a great importance comes to being brassinosteroid, whose presence is quite ample in plant kingdom with different analog compounds being synthetized for commercial usage (Cortes et al., 2003). Synthetic brassinosteroids, such as BIOBRAS 16®, are being tested and evaluated with regards to its efficacy in plant growth and development. The effects of brassinosteroids on growth and initial development of plants demonstrate a synergism trend with other phytohormones, mainly auxins (Hardtke et al., 2007).

Plants respond to abiotic and biotic factors in the environment. These include heavy metals action, wounding, drought, high salt, and changes in temperature and light, and pathogen and pest attack. Abiotic stress leads to morphological, physiological, biochemical and molecular changes. Drought, salinity, extreme temperatures and oxidative stress are often interconnected, and may induce similar cellular damage. Brassinosteroids can act efficiently in plants as immunomodulators when applied at the appropriate concentration and at the correct stage of plant development. BRs are implicated in plant responses to abiotic environmental stresses and to undergo profound changes in plants interacting with bacterial, fungal and viral pathogens (Badrzej & Hayat, 2009).

Souza et al. (2011) verified that micro-propagated blueberry plants evidenced a larger initial vegetative growth—a motive for tissues rejuvenation triggered by the micropropagation technique. Cüce et al. (2013), by making use of different concentrations of IBA, observed that smaller doses of auxin (0.5 mg $L^{-1}$) induced *in vitro* rooting of blueberry. The aforementioned authors also came up with a more pronounced aerial part development whenever 1.0 mg $L^{-1}$ of zeatin was utilized along with 0.1 mg $L^{-1}$ of IBA in light of a bigger number of leaves and proliferation of multiple branches taking place in the tissue culture supplemented with 2.0 mg $L^{-1}$ of zeatin plus 0.1 mg $L^{-1}$ of IBA.

Müssig et al. (2003), by testing different doses of 24-epicastasterose and 24-epibrassinolideo brassinosteroids applied in mutant Arabidopsis plants with deficiency of this hormone, drew the conclusion that low concentrations promote radicular elongation of such plants. Thus, the use of brassinosteroids might favor radicular development in such a way as to better its efficiency throughout the *in vitro* development process of the plants.

In view of the aforementioned micropropagation problem, the aim of the current manuscript was to assess the effect of different concentrations of BIOBRAS 16® in conjunction with IBA on the *in vitro* rooting of blueberry.

## 2. Material and Methods

The experiment was carried out along with explants extracted from stem cuttings of blueberry (*Vaccinium ashei* Reade), comprising 1 to 2 buds with 1 to 1.5-cm length priorly grown *in vitro* for micropropagation.

The culture tissue utilized herein was Wood Plant Medium (WPM) as preconized by Lloyd and McCown (1991) in the face of a supplementation with saccharoses (3%), myo-inositol (100 mg $L^{-1}$), agar (6 g $L^{-1}$) plus BIOBRAS 16® (0.1; 0.3 and 0.5 mg $L^{-1}$) and IBA (1.0; 3.0 and 5.0 mg $L^{-1}$) in accordance with each treatment. The medium pH was regulated at 5.3 shortly before addition of agar, taking into account a further dose of 30 mL of such a medium in glassy containers for *in vitro* cultivation so that afterwards they could have been auto-clavated at 120 $^{o}$C for roughly 20 minutes.

The containers were kept at a growth chamber with photoperiod featured by 16-hour light along with 8-hour dark, photons flux density of 27 μmol $M^{-2}$ $s^{-2}$ and air temperature of 25±2 ºC. After completion of 82 days of *in vitro* cultivation, the developed explants were assessed as to CP (%), CD (mm), RP (%), NS, NL, SL (mm), RL (mm) and FMS (g). For all variables scrutinized, their mean values from each replication were obtained and subjected to statistical analyses.

In order to test statistical hypotheses, we adopted a completely randomized design in a double factorial scheme (3 × 3) with the first factor considered as concentrations of BIOBRAS ® (0.1; 0.3 and 0.5 mg $L^{-1}$) and the second factor as concentrations of IBA (1.0; 3.0 and 5.0 mg $L^{-1}$), having been employed for each single treatment four containers with 5 explants. Treatment averages were compared by means of Tukey test at a $p \leq 0.05$. Analyses were performed by making use of AgroEstat Statistical Program (Barbosa & Maldonado Junior, 2015).

## 3. Results and Discussion

The mean values of CP, CD, RP, NS and SL are illustrated in Table 1. For the CP response variable, we came to the conclusion that at any BIOBRAS 16® concentration when in conjunction with concentrations of IBA corresponding to 3.0 and 5.0 mg $L^{-1}$ there was a higher percentage of callus formation. Radmann et al. (2002), by testing indoleacetic acid (IAA) plus IBA aiming at in vitro rooting of M-9 apple rootstock, verified that high concentrations of such a phytohormone retard rooting itself and cause induction to occur throughout the callus formation at the basis of explants. Such outcomes corroborate those found for blueberry (Table 1), from which

treatments comprising 0.3 and 0.5 mg $L^{-1}$ of Biobras ® combined with the highest IBA concentrations of either 3.0 or 5.0 mg $L^{-1}$ were conducive to the highest percentage of callus formation.

In view of CD of blueberry (Table 1), the highest concentrations of IAA under scrutiny (3.0 and 5.0 mg $L^{-1}$) irrespective of the BIOBRAS 16® concentrations (0.1, 0.3, and 0.5 mg $L^{-1}$) were those ones which culminated in the highest CD values. Therefore, higher concentrations favored cellular division and also promoted formation of new cells.

The RP of *in vitro* micro-propagated blueberry (Table 1) evidenced a beneficial responsiveness under treatments of 3.0 and 5.0 mg $L^{-1}$ of AIA along with 0.1, 0.3, and 0.5 mg $L^{-1}$ of BIOBRAS 16®. It is quite well expected that in so far as auxin concentration rises up to a favorable physiological threshold stimulus for development of radicular system will be taking place once such a phytohormone turns out to be conducive to formation of roots (Mihaljević & Salopek-Sondi, 2012; Taiz & Zeiger, 2017). This fact is evidenced as a function of resilience inherent to cells that preserve their capacity of dividing and in turn originating a radicular meristem in analogous manner with regards to formation of lateral roots (Smet et al., 2006; Kerbauy, 2008; Taiz & Zaiger, 2017). Capacity of rooting has a strong relationship with individual morphology of roots, as well as growth potential and architecture of radicular system (Baba et al., 2018).

Sousa et al. (2017), by making use of IBA to stimulate *in vitro* rooting of *Anacardium othonianum* Rizz, observed that under a high concentration of IBA corresponding to 4 mg $L^{-1}$ a conspicuous stimulus for root length was envisioned. Conversely, in the light of a low concentration of IAA (1.0 mg $L^{-1}$) low RE values were found. In addition, under the influence of low concentrations of auxin, radicular system takes a long time to express any significant stimulus for development. Similar findings were reported by Santos et al. (2006) for micropropagation of *Caryocar brasiliense*.

Table 1. Callus percentage (CP), callus diameter (CD), rooting percentage (RP), number of shoots (NS) and shoot length (SL) for micro-propagated blueberry subjected to distinct concentrations and combinations of IBA plus BIOBRAS 16®

| IBA (mg $L^{-1}$) | BIOBRAS 16® (mg $L^{-1}$) | | |
|---|---|---|---|
| | 0.1 | 0.3 | 0.5 |
| | ---------- Callus Percentage (CP) ---------- | | |
| 1.0 | 56 bB | 81 bA | 88 bA |
| 3.0 | 94 aB | 100 aA | 100 aA |
| 5.0 | 81 aB | 94 aA | 100 aA |
| CV (%) | 20.95 | | |
| | ---------- Callus Diameter (CD, mm) ---------- | | |
| 1.0 | 4.84 bA | 5.06 bA | 7.41 bA |
| 3.0 | 8.86 aA | 8.19 aA | 7.80 aA |
| 5.0 | 6.33 aA | 8.58 aA | 7.72 aA |
| CV (%) | 21.3 | | |
| | ---------- Rooting Percentage (RP) ---------- | | |
| 1.0 | 25 bB | 66 bA | 25 bB |
| 3.0 | 50 aB | 81 aA | 69 abAB |
| 5.0 | 69 aB | 75 aA | 81 aA |
| CV (%) | 20.5 | | |
| | ---------- Number of Shoots (NS) ---------- | | |
| 1.0 | 1.25 | 1.88 | 1.31 |
| 3.0 | 1.00 | 1.13 | 1.00 |
| 5.0 | 1.06 | 1.00 | 1.06 |
| CV (%) | $37.63^{N.S.}$ | | |
| | ---------- Shoot Length (SL, mm) ---------- | | |
| 1.0 | 32.83 | 34.42 | 27.75 |
| 3.0 | 35.88 | 49.00 | 42.88 |
| 5.0 | 47.44 | 34.80 | 29.50 |
| CV (%) | $30.57^{N.S.}$ | | |

*Note*. Averages followed by the same small letters in the column, as well as capital letters in the line did not significantly differ among themselves by means of the Tukey test at 5% reliability level. $^{N.S.}$: Non-significant.

The rooting of explants multiplied *in vitro* comes to being a pre-requisite for any micropropagation protocol; this is because it impinges directly upon acclimatization and installation of seedlings in the soil (Patti et al., 2006; Baba et al., 2018). Several factors govern rooting responsiveness, such as exogen and endogen factors like ethylene for instance (Ayub et al., 2017), mainly in consideration for inducing formation of roots in species that present difficulties for rooting (Hartmann et al., 2002; Assis & Teixeira, 1998).

NS and SL of blueberry (Table 1) as a function of combinations of IAA and BIOBRAS 16® did not show any significant statistical differences under the influence of the treatments applied herein. This might be explained by the lack of cytokinin added to culture tissue, counting only on regulators involved in the radicular development process. In accordance with Lopes et al. (2012), benzylaminopurine (BAP) and kinetin (KIN) are the cytokinins that lead to best results concerning the proliferation of shootings (NS) in buds of *Jatropha curcas* L. The utilization of BAP galvanizes formation of aerial part of the plants; however, it might be quite conducive to distinct physiological responses as a function of the specie at issue, highlighting such an increment in formation process of aerial part of plants grown *in vitro* when it comes to *Jatropha curcas* L.

It was possible to notice that RL of blueberry showed a positive responsiveness at IAA concentrations of 3.0 and 5.0 mg $L^{-1}$ as opposed to IAA concentration of 1.0 mg $L^{-1}$, irrespective of the Biobras® concentration (Table 2). Auxin is a hormone supposedly prone to induce formation of roots, whose activity increases substantially as a function of rises in concentration of such a phytohormone up to a certain plateau. Once reached its maximum peak, an opposite physiological impact on rooting might be observed (Taiz & Zeiger, 2017). Although non-evidences dealing with increments in concentration of brassinosteroids meant to influence root length were reported, such a hormone plays a role in biological activity of aquaporins present in the plasmatic membrane, resulting in increases in permeability to water within the cells (Morillon et al., 2001); which might potentialize then the effect of auxin on enlargement of cells plus growth of plant tissues, including the roots. Parallelly, in the lieu of longer roots such an enlargement significantly contributes to promote an overall increase in root length (Baba et al., 2018).

Table 2. Root length (RL), number of leaves (NL) and fresh mass (FM) of micro-propagated blueberry subjected to distinct concentrations and combinations of IBA and BIOBRAS 16®

| IBA (mg $L^{-1}$) | BIOBRAS 16® (mg $L^{-1}$) | | |
|---|---|---|---|
| | 0.1 | 0.3 | 0.5 |
| | ---------- Root Length (RL, mm) ---------- | | |
| 1.0 | 2.00 bA | 3.12 bA | 1.81 bB |
| 3.0 | 6.19 aA | 7.00 aA | 5.63 aA |
| 5.0 | 9.06 aA | 11.25 aA | 6.88 aB |
| CV (%) | 76.82 | | |
| | ---------- Number of Leaves (NL) ---------- | | |
| 1.0 | 12.44 aA | 12.75 aA | 14.19 aA |
| 3.0 | 11.42 aA | 11.84 aA | 10.69 abA |
| 5.0 | 8.44 bA | 8.31 bA | 8.38 bA |
| CV (%) | 21.54 | | |
| | ---------- Fresh Mass (FM, g) ---------- | | |
| 1.0 | 0.21 bA | 0.16 bA | 0.20 bA |
| 3.0 | 0.25 aA | 0,28 aA | 0.24 aA |
| 5.0 | 0.30 aA | 0.22 abA | 0.21 abA |
| CV (%) | 22.07 | | |

*Note*. Averages followed by the same small letters in the column, as well as capital letters in the line did not significantly differ among themselves by means of the Tukey test at 5% reliability level. $^{N.S.}$: Non-significant.

By scrutinizing number of leaves (NL) of micro-propagated blueberry (Table 2), we detected that NL plummeted regardless of the IBA concentration adopted in so far as IBA concentration increased. It is quite expected that a detrimental impact on leaf area will be seen whenever IBA concentration increases (Taiz & Zeiger, 2017).

As to fresh mass (FM) of micro-propagated of blueberry (Table 2), outcomes demonstrated that at the lowest concentration of BIOBRAS 16® (0.1 mg $L^{-1}$) in conjunction with IBA concentrations of 3.0 and 5.0 mg $L^{-1}$ led to

the highest FM. On the other hand, whenever 0.3 and 0.5 mg $L^{-1}$ concentrations of BIOBRAS 16® plus a 3.0 mg $L^{-1}$ concentration of IBA were achieved FM reached its peak.

When it comes to micropropagation irrespective of the species under scrutiny, it is quite pivotal to take into consideration the formation of roots throughout the seedling's establishment. The process of *in vitro* rooting per se is complex because it encompasses physiological, biochemical and biological factors that alter and intricately interact with one another under the interference of external factors (Meira, 1999: Baba et al., 2018), being also remarkably governed by genetic variability of the species along with cultivars (Schmildt et al., 2010). For blueberry in particular, it is possible to make use of IBA in association with BIOBRAS 16® aiming at induction of *in vitro* rooting (Tables 1 and 2). Nevertheless, it is opportune to stand out that a satisfactory root development in the face of micropropagation does not only depend on rooting percentage, but also on root number and quality of formed roots (George et al., 2008; Baba et al., 2018).

The foliar application of Biobras-16 during the acclimatization of BRS Guaraçá seedlings multiplied via mini-cuttings produces significant positive effects on shoot and root growth; concentrations between 0.3 and 0.6 mg $L^{-1}$ enable the production of better-quality seedlings in less time in order to improve soil fertility and agronomic efficiency of nutrients (Arantes et al., 2020).

The use of the brassinosteroids analogue stimulated a higher production of normal seedlings of *Acrocomia aculeata* but did not promote an increase in the germination rate. During the acclimatization phase, the application of arbuscular mycorrhizal fungi (AMF) integrated to rhizobacteria and brassinosteroid analogue was not effective on the plant aerial growth but favored a higher root system development (Borcioni, 2012).

## 4. Conclusions

The utilization of a 0.3 mg $L^{-1}$ concentration of BIOBRAS 16® in conjunction with 3.0 and 5.0 mg $L^{-1}$ concentrations of IBA promoted the highest possible percentage of formation of roots for blueberry. Such a combination contributes to enhance growth of roots, number of leaves and fresh mass of micro-propagated blueberry.

The outcomes obtained from laboratory assessments were not yet validated under field specific-sites. Nevertheless, further field experiments are supposed to be carried out in the near future so that transferability of our current findings might be applicable to multiple field sites in Southern Brazil.

## References

Arantes, M. B. S., Marinho, C. S., Gomes, M. M. A., Santos, R. F., Galvão, S. P., & Vaz, G. P. (2020). Brassinosteroid accelerates the growth of *Psidium* hybrid during acclimatization of seedlings obtained from mini-cuttings. *Pesquisa Agropecuária Tropical, 50*, e64743. https://doi.org/10.1590/1983-40632020v5064743

Araujo, A. G. de, Pasqual, M., Silva, B. A. da, Villa, F., Rocha, H. S., & Costa, F. C. (2006). Propagação in vitro de plântulas de orquídea em diferentes meios de cultura e concentrações de citocinina. *Plant Cell and Culture Micropropagation, 2*, 68-73.

Assis, T. F., & Teixeira, S. L. (1998). Enraizamento de plantas lenhosas. *Cultura de tecidos e transformação genética de plantas* (pp. 261-296). Brasília: Embrapa CNPH.

Ayub, R. A., Pasqualini, A. P. A., Santos, J. N., & Botelho, R. V. (2017). Blueberry (*Vaccinium ashei* Reade) cv. Brightwell *in vitro* establishment with silver thiosulfate. *Plant Cell Culture & Micropropagation, 13*(1), 1-6.

Baba, T., Nakaba, S., Noma, S., Funada, R., & Ban, T. (2018). Heterorhizy and fine root architecture of rabbit eye blueberry (*Vaccinium virgatum*) softwood-cuttings. *Journal of Plant Research, 131*, 271-284. https://doi.org/10.1007/s10265-017-1000-y

Badrzej, B., & Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiology and Biochemistry, 47*(1), 1-8. https://doi.org/10.1016/j.plaphy.2008.10.002

Barbosa, J. C., & Maldonado Junior, W. (2015). *AgroEstat—Sistema para análises estatísticas de ensaios agronômicos* (p. 396). Jaboticabal: FCAV/UNESP.

Borcioni, E. (2012). *Subsídios à implementação de sistemas de cultivo de Acrocomia aculeata (Jacq) Lood Ex. Mart (Arecaceae)* (p. 113, PhD Thesis in Agronomy, Crop Production, Federal University of Paraná, Brazil).

Cantuarias-Avilés, T. (2010). Cultivo do mirtileiro (*Vaccinium* sp.). *Série Produtor Rural, 48* (p. 38). Piracicaba: ESALQ.

Cantuarias-Avilés, T., Silva, S. R., Medina, R. B., Moraes, A. F. G., & Alberti, M. F. (2014). Cultivo do mirtilo: Atualizações e desempenho inicial de variedades de baixa exigência em frio no estado de São Paulo. *Revista Brasileira de Fruticultura, 36*(1), 139-147. https://doi.org/10.1590/0100-2945-453/13

Cortes, P. A., Salgado, T. M. T., León, T. C., & Larqué-Saavedra, A. (2003). Brassinosteroid effects on the precocity and yield of cladodes of cactus pear (*Opuntia ficus-indica* (L.) Mill.). *Scientia Horticulturae, 97*(1), 65-73. https://doi.org/10.1016/S0304-4238(02)00080-8

Cüce, M., Bektas, E., & Sokmen, A. (2013). Micropropagation of *Vaccinium arctostaphylos* L. via lateral-bud culture. *Turkish Journal of Agriculture and Forestry, 37*, 40-44.

Erig, A. C., & Schuch, M. W. (2005). Estabelecimento in vitro de mirtilo a partir de segmentos nodais. *Scientia Agrária, 6*(1-2), 91-96. https://doi.org/10.5380/rsa.v6i1.4603

George, E. F., Hall, M. A., & de Klerk, G. J. (2008). *Plant propagation by tissue culture: The background* (Vol. 1, 3rd ed.). Dordrecht: Springer.

Hardtke, C. S., Dorcey, E., Osmont, K. S., & Sibout, R. (2007). Phytohormo0ne Collaboration: Zooming in on Auxin-Brassinosteroid Interactions. *Trends Cell Biology, 17*, 485-492. https://doi.org/10.1016/j.tcb.2007.08.003

Hartmann, H. T., Kester, D. E., Davies Junior, F. T., & Geneve, R. L. (2002). *Plant propagation: Principles and practices* (7th ed.). New Jersey: Prentice Hall.

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2019). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition, 11*(2), 1-13. https://doi.org/10.1093/advances/nmz065

Kerbauy, G. B. (2008). *Fisiologia vegetal* (2nd ed., p. 431). Rio de Janeiro: Guanabara Koogan.

Lloyd, G., & Mccown, B. (1981). Use of microculture for production and improvement of *Rhododendron* spp. *HortScience, 15*(3), 416.

Meira, Z. (1999). Developmental and structural patterns of in vitro plants. In W. Y. Soh & S. S. Bhojwani (Eds.), *Morphogenesis in plant tissue cultures* (pp. 235-254). Dordrecht: Kluwer Academic Publishers.

Mihaljević, S., & Salopek-Sondi, B. (2012). Alanine conjugate of indole3-butyric acid improves rooting of highbush blueberries. *Plant Soil Environment, 58*, 236-241. https://doi.org/10.17221/34/2012-PSE

Morillon, R., Catterou, M., Sangwan, R. S., Sangwan, B. S., & Lassalles, J. P. (2001). Brassinolide may control aquaporin activities in *Arabidopsis thaliana*. *Planta, 212*(2), 199-204. https://doi.org/10.1007/s004250000379

Müssig, C., Shin, G. H., & Altmann, T. (2003). Brassinosteroids promote root growth in Arabidopsis. *Plant Physiology, 133*(3), 1261-1271. https://doi.org/10.1104/pp.103.028662

Pasqualini, A. P. de A., Santos, J. N. dos, & Ayub, R. A. (2016). Behavior and viability of blueberry seeds through germination and tetrazolium test. *Advances in Bioscience and Biotechnology, 7*(1), 11-18. https://doi.org/10.4236/abb.2016.71002

Pati, P. K., Rath, S. P., Sharma, M., Sood, A., & Ahuja, P. S. (2006). *In vitro* propagation of rose: A review. *Biotechnology Advances, 24*(1), 94-114. https://doi.org/10.1016/j.biotechadv.2005.07.001

Peña, M. L. P., Gubert, C., Tagliani, M. C., Bueno, P. M. C., & Biasi, L. A. (2012). Concentrações e formas de aplicação do ácido indolbutírico na propagação por estaquia dos mirtileiro cvs. *Flórida e Climax. Semina: Ciências Agrárias, 33*(1), 57-64. https://doi.org/10.5433/1679-0359.2012v33n1p57

Radmann, E. B., Fachinello, J. C., & Peters, J. A. (2002). Efeito de auxinas e condições de cultivo no enraizamento *in vitro* de porta-enxertos de macieira 'M-9'. *Revista Brasileira de Fruticultura, 24*(3), 624-628. https://doi.org/10.1590/S0100-29452002000300011

Santos, B. R., Paiva, R., Nogueira, R. C., Oliveira, L. M., Silva, D. P. C., Martinotto, C., **Soares, F.P. &** Paiva, P. D. O. (2006). Micropropagação de pequizeiro (*Caryocar brasiliense* Camb.). *Revista Brasileira de Fruticultura, 28*(2), 293-296. https://doi.org/10.1590/S0100-29452006000200031

Schmildt, E. R., Amaral, J. A. T., Schmildt, O., Coelho, R. I., Rabello, W. S., & Martins Filho, S. (2010). Níveis de ácido indolbutírico (AIB) no enraizamento *in vitro* de microestacas de mamoeiro 'Tainung 01'. *Acta Scientiarum. Agronomy, 32*(1), 125-129. https://doi.org/10.4025/actasciagron.v32i1.866

Schuch, M. W., Rossi, A., Damiani, C. R., & Soares, G. C. (2007). AIB e substrato na produção de mudas de mirtilo cv. "Climax" através de microestaquia. *Ciência Rural, 37*(5), 1446-1449. https://doi.org/10.1590/S0103-84782007000500036

Smet, I., Vanneste, S., Inzé, D., Beeckman, T. (2006). Lateral root initiation or the birth of a new meristema. *Plant Molecular Biology, 60*(6), 871-887. https://doi.org/10.1007/s11103-005-4547-2

Sousa, I. D., Sousa, J. B., Pereira, F. D., Santana, J. G., Neto, A. R., & Assis, E. S. (2017). Composition of the cultivation medium for the production of microplants of Cerrado-tree cashew. *Revista Científica, 5*(1).

Souza, A. L. K., Schuch, M. W., Antunes, L. E. C., Schmitz, J. D., Pasa, M. S., Camargo, S. S., & Carra, B. (2011). Desempenho de mudas de mirtilo obtidas por micropropagação ou estaquia. *Pesquisa Agropecuária Brasileira, 46*(8), 868-874. https://doi.org/10.1590/S0100-204X2011000800012

Taiz, L., Zeiger, E., Moller, I., & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal* (6th ed., p. 888). Porto Alegre: Artmed.